the phase diagram itself. Brewster angle microscopy allows one to image the domains in a Langmuir monolayer with and without the probe molecules to directly test their effect. The combined Brewster angle (BAM)/ fluorescence microscope allows us to image simultaneously with the two techniques exactly the same domains in the Langmuir film. In general, the images taken by the two microscopes compare well. Comparison of the techniques can then make it easier to correlate the different domain properties leading to contrast in the two techniques. Some types of domains may however be much more evident with BAM than with FM.

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Partitioning of Solutes in Lipid Multilayers
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When placed in water, lipid molecules form multilamellar vesicles (MLVs) in which lipid layers are separated by water regions. These interlamellar water regions have thicknesses on the order of 1 to 10 nanometers depending on lipid type. What happens if water contains buffer molecules? Will buffer molecules be taken inside the MLVs? If yes, then to what extent? These questions arise because the physical properties of water regions next to lipid membranes have been shown to differ from bulk water [1]. We would like to know how buffer molecules and other solutes partition between MLVs and the outer solution. To determine this partitioning, we use lipid membranes that sink in pure water but float in buffer solutions of certain concentrations. We then find the exact concentration for which the mass density of the solution matches that of the MLVs. This density matching allows us to calculate the ratio between water and buffer molecules present inside MLVs by using data from small-angle x-ray scattering. For KCl solutions, as well as for solutions of common buffers, we find that solutes are excluded from the interlamellar water regions creating a solute deficit inside the MLVs. [1] Petrache et al., Biophys. J. 86, 2004.

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Coarsening Dynamics of Domains in Lipid Membranes
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We investigate diffusion and growth of liquid domains within membranes of giant unilamellar vesicles (GUVs) composed of ternary lipid mixtures. Domains appear after a temperature quench, when the membrane is cooled through a miscibility phase transition such that coexisting liquid phases form. In membranes quenched far from a miscibility critical point, circular domains nucleate and then progress within seconds to ''late stage'' coarsening in which domains grow via two mechanisms: (1) collision and coalescence of liquid domains, and (2) Ostwald ripening. Both mechanisms are expected to yield the same growth exponent, alpha = 1/3, where domain radius grows as time raised to a power of alpha. In membranes close to a miscibility critical point, the two liquid phases in the membrane are bicontinuous. A quench near the critical composition results in rapid changes in morphology of elongated domains; theory and simulation predict alpha = 1/2. Here we measure growth exponents for micron-scale domains in vesicles with diameters between 80 microns and 250 microns. The vesicles undergo a fast temperature quench and then are observed at roughly constant temperature. We find an exponent of alpha = 0.28 ± 0.05 far from the critical point and alpha = 0.50 ± 0.16 close to the critical point, in good agreement with theory.

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Molecular Dynamics Simulation, 31P-NMR Spectroscopy, and Microelectrophoretic Studies of Cardiolipin Bilayers
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Molecular dynamics (MD) simulations of tetramyristoyl cardiolipin (TMCL) and tetraoleoyl cardiolipin (TOCL) were carried out with the newly developed CHARMM C36 lipid force field (FF), and with head group charges q= −1 and −2. The surface areas per lipid, AL, for q=-2 are 127.1 ± 0.4 Å2 for TOCL and 113.2 ± 0.2 Å2 for TMCL. To validate the simulation results, 70% TOCL + 30% POPC bilayer was prepared both in simulation and NMR experiment. The order parameters of POPC from simulations were higher than those from experiment. Sodium-lipid van der Waals interactions were slightly reduced through CHARMM NBFIX. This change of parameters led to 5% increase in surface area/lipid, and substantially improved agreement with experiment for q = -2 TOCL. The net charge of cardiolipin at neutral pH has been the subject of debate. Microelectrophoresis and 31P-NMR spectroscopy experiments were carried out, and indicate that the net charge is −2.

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Diacylglycerol Pyrophosphate: Novel Signaling Lipid with Undetermined Function
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Diacylglycerol pyrophosphate (DGPP) is an enigmatic phospholipid that has not been fully characterized. DGPP is a phosphorylated form of phosphatidic acid (PA) found in yeast and plants and has unique chemical features, namely it carries a pyrophosphate headgroup. PA is a signaling lipid important for many cellular processes including membrane bending/destabilization, stress response in plants, and protein docking to the membrane. PA-protein interaction can be explained on the basis of the recently formulated electrostatic/hydrogen bond switch model. PA signaling in plants is attenuated by its metabolism to DGPP which is thus found to increase in concentration following abiotic or biotic stress conditions (drought, salinity, cold, etc.). In order to understand the function of this increase in DGPP upon an increase of PA, we need to know the physicochemical properties of this enigmatic lipid first. We therefore set out to determine the phase behavior and ionization properties of DGPP in model membrane systems in the presence of divalent cations and lysine rich peptides. In our previous analyses of DGPP, the phosphomonoester was shown to be able to act as an electrostatic/hydrogen bond switch like PA. These results are now extended to more physiologically relevant conditions and the presence of transmembrane peptides. This work was supported by a Farris Family fellowship to EEK and by Kent State University.

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Evaluating a Method for Producing Asymmetric Giant Unilamellar Vesicles
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Giant unilamellar vesicles (GUVs) are important model systems for investigating physical properties of lipid bilayers. Recently, a method was developed called continuous droplet interface crossing encapsulation (cDICE)[1], which creates GUVs by passing a stream of droplets through an oil water interface. Several methods, such as electroformation and gentle hydration, exist to produce GUVs. In comparison, cDICE is better suited to incorporating large fractions of charged lipids in membranes, to creating vesicles in a high-salt environment, and to encapsulating large objects within vesicles. All of these features are important for achieving larger goals of understanding both the underlying physics and the biological role of lipid bilayers. Here, we investigate an extension of the cDICE method for producing asymmetric GUVs, which are GUVs with inner and outer leaflets of different compositions. We tested asymmetry by fluorescence quenching experiments. Our goal is to create a model system mimicking the asymmetry found in biological membranes.
1. Abkarian, M, Louiseau, E, and Massiera, G. ***Soft Matter***, 2011, **7**, 4610-4614

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Membrane Binding Kinetics of Endophilin N-BAR Domain
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It has been suggested that the crescent shape and the presence of amphipathic helices on N-BAR domain dimers explain endophilin's involvement in endocytosis by scaffolding and/or hydrophobic insertion mechanisms. However, studies of these mechanisms in proteins with membrane curvature sensing/ generation properties thus far have mainly focused on thermodynamic equilibrium conditions. Bridging the connection between thermodynamics to highly dynamic cellular environments calls for studies exploring binding kinetic aspects.